Sex Differences in the Densities of Epidermal Langerhans Cells of the Mouse

Yoh-ichi Koyama, Ph.D., Shizuko Nagao, B.H., Koji Ohashi, B.H., Hisahide Takahashi, Ph.D., and Tohru Marunouchi, Ph.D.

Division of Cell Biology (YK, KO, TM) and Center of Laboratory Animal (SN, HT), Institute for Comprehensive Medical Science, Fujita-Gakuen Health University, Toyoake, Aichi, Japan

Cutaneous immune reactions are known to show sexual dimorphism. Langerhans cells (LCs) are bone marrow–derived immune cells in the epidermis and are essential to immune reactions in the skin. In the present research, a study was made of the differences in LC density of male and female mice. Epidermal sheets were separated from the skin of the glabrous part of hind limbs and ears of specific pathogen-free (SPF) mice by ethylenediaminetetraacetic acid (EDTA) treatment and stained for adenosine triphosphatase (ATPase) activity. The density of LCs of hind limb epidermis in male C57BL/6 (823 ± 20/mm^2) and BALB/c (1689 ± 66/mm^2) mice was significantly less than that in females (1363 ± 52/mm^2, $p < 0.001$; 2249 ± 105/mm^2, $p < 0.001$, respectively). Langerhans cell density in the ears of male C57BL/6 (465 ± 24/mm^2) mice was also significantly less than that in females (542 ± 17/mm^2, $p < 0.02$). Although ovariectomy failed to bring about any change in the LC density of hind limb epidermis in female C57BL/6 mice, the LC density in male C57BL/6 mice increased significantly at 4 weeks following orchiectomy (sham operation, 564 ± 27/mm^2; castration, 1179 ± 49/mm^2, $p < 0.001$). These results indicate that mouse epidermal LC density depends on sex, i.e., male mice have fewer LCs than female mice. The reduction in LC density in males may possibly be caused by the testis. *J Invest Dermatol 88: 541–544, 1987*

Langerhans cells (LCs) were first discovered by Paul Langerhans in 1868 in a gold chloride-stained preparation of skin [1]. Although their origin and functions are controversial, it is now well established that LCs are bone marrow–derived immune cells in the epidermis. They express Ia antigens and are essential to the induction of T-cell response in the self-nonself discrimination system (reviewed in [2,3]). For example, depletion of LCs from the epidermal cell population markedly reduces T-cell response, suggesting that their density in the epidermis is important for immune reactions in the skin [4–6].

Sexual dimorphic phenomena in both humoral and cellular immunity have been observed in various experimental systems [7–9]. In most cases, females show higher immune responses than males, and orchiectomy enhances immune activity of males. Graff, Lappé, and Snell reported that females reject skin allografts more consistently and rapidly than males, and that orchiectomy results in accelerated rejection of skin allografts in males [8].

Assuming that LCs play a critical role in skin graft rejection, the density and functions of LCs may reflect sexual dimorphism and be influenced by castration. Although there are a number of reports on epidermal LC density in humans [10,11], guinea pigs [12], and mice [4], sex differences have yet to be observed in any of these species. In the present research, an investigation was made of the densities of LCs in the hind limb and ear skin of mice to determine whether LC density shows sex differences. To examine the relationship between LC density and gonads, orchiectomy and ovariectomy were performed. Since adenosine triphosphatase (ATPase) activity is widely used as a highly reliable marker of LCs [3], ethylenediaminetetraacetic acid (EDTA)-separated epidermal sheets were stained for ATPase activity and the number of LCs was counted.

Manuscript received April 17, 1986; accepted for publication August 15, 1986.

This work was supported in part by a grant-in-aid for scientific research from Fujita-Gakuen Health University.

Reprint requests to: Yoh-ichi Koyama, Ph.D., Division of Cell Biology, Institute for Comprehensive Medical Science, Fujita-Gakuen Health University, Toyoake, Aichi 470-11, Japan.

Abbreviations:
LC: Langerhans cell
PBS: phosphate-buffered saline
SPF: specific antigen-free

MATERIALS AND METHODS

Animals Eight-week-old, specific pathogen-free (SPF) C57BL/6 (C57BL/6NCrj) and BALB/c (BALB/cAnNCrj) mice were purchased from Charles River Japan Inc. The mice were used as soon as possible following their arrival at our laboratory or kept in a clean condition following operation.

Langerhans Cells of Hind Limb Skin Adenosine triphosphatase staining was carried out according to the method of Baker and Habowsky with some modification [13]. Skin specimens from the glabrous part of both hind limbs, proximal to the footpad, were incubated for 2 h at 37°C in a freshly prepared solution of 10 mM Na_2-EDTA·$2H_2O$ in phosphate-buffered saline (PBS) (NaCl 6.83 g, KCl 0.2 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.2 g per 1 liter). Epidermal sheets were separated from the dermis with fine forceps. After brief washing in PBS at room temperature, the specimens were fixed with 2% paraformaldehyde in 0.075 M cacodylate-HCl (pH 7.3) for 20 min at 4°C. The epidermal sheets were rinsed in 0.15 M NaCl at 4°C (10 min × 3) and allowed to react with an ATP-Pb solution for 15 min at 37°C. The ATP-Pb solution consisted of 42 ml of 0.2 M Trismal buffer (pH 7.3, 8.55% sucrose), 3 ml of 0.06 M $Pb(NO_3)_2$, 5 ml of 0.41 M $MgSO_4·7H_2O$. Ten milligrams of Na_2-ATP·$3H_2O$ (WAKO, 017-

099973) were added to the solution just before use. After being washed in 0.15 M NaCl at 4°C (10 min × 3), the reaction products were rendered visible by incubation in a 1% solution of ammonium sulfide for 20 min at 4°C. The epidermal sheets were washed in 0.15 M NaCl at 4°C (5 min × 3) and mounted on glass slides in glycerin:PBS (9:1).

Langerhans Cells of Dorsal Ear Skin Both ears were cut off and cut up into square pieces (4 × 4 mm) with a knife. The dorsal skin was separated from underlying cartilage with fine forceps. For ATPase staining of ear epidermis, the method was modified in some respects to improve the reaction. $Na_2ADP{\cdot}2H_2O$ (WAKO, 306-50501) was used in place of ATP according to Chaker et al [14]. Skin pieces were treated with EDTA for 3 h instead of 2 h, and the concentration of the $MgSO_4{\cdot}7H_2O$ solution added to the ADP-Pb solution was increased to 1.93 M.

Enumeration of LCs The LCs were counted with a reticle at ×200. One field outlined by the reticle corresponded to an area of 0.016 mm^2. One skin piece was obtained from each hind limb and ear, and the number of LCs was counted in 2 randomly selected fields of each sample (a total area of 0.032 mm^2). The mean of 4 values obtained for both hind limbs and ears of each mouse was calculated and used as the LC density of each animal for calculation of the mean ± SEM of each group. Cell counting was carried out by the blind test.

Castration Eight-week-old C57BL/6 mice were ovariectomized or orchiectomized under Nembutal anesthesia. The number of LCs of the hind limb skin was counted at 4 weeks following the operation. Control animals were sham operated.

Statistical Methods The Student *t*-test was used to assess differences in LC densities. A p value of less than 0.05 was considered significant.

RESULTS

Langerhans Cells of Hind Limb Skin Adenosine triphosphatase staining of the epidermal sheet indicated the LCs to be reaction-positive. The LCs in both male (Fig 1) and female (Fig 2) C57BL/6 mice showed regular distribution, but cell density was less and dendrites were longer in males than in females (males, 823 ± 20/mm^2; females, 1363 ± 52/mm^2, Table I). In BALB/c mice, sex differences in LC density were also significant (males, 1689 ± 66/mm^2; females, 2249 ± 105/mm^2, Table I). Although the LCs were regularly spaced as in C57BL/6, the dendrites were rather short and differences in dendrite length were not apparent between the two sexes. Male/female LC ratios were 0.63 for C57BL/6 and 0.75 for BALB/c.

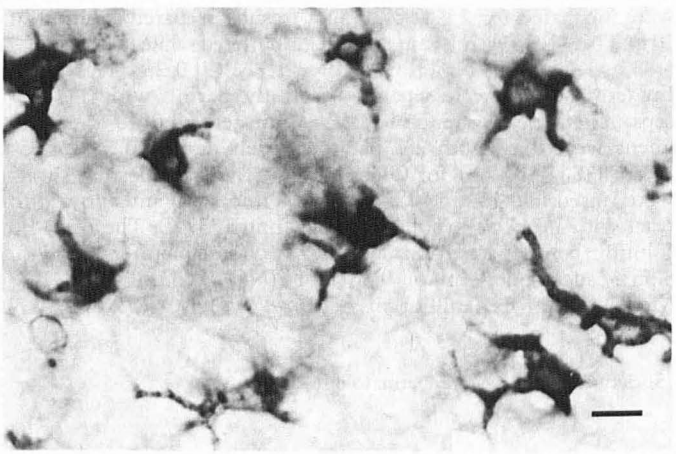

Figure 1. Epidermal sheet from the hind limb skin of a male C57BL/6 mouse showing LCs stained for ATPase activity. *Bar* = 10 μm.

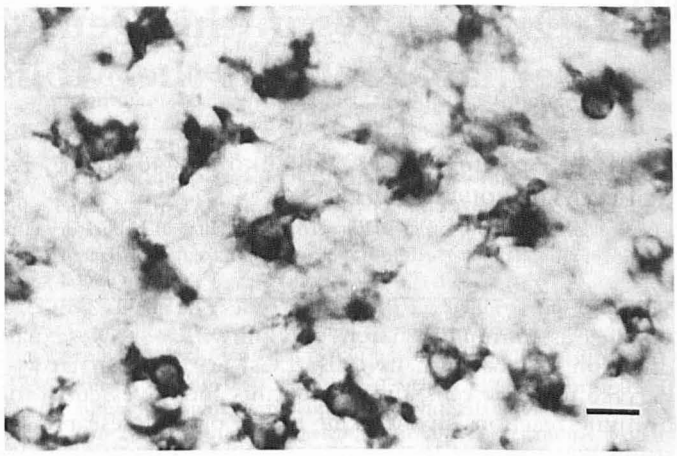

Figure 2. Epidermal sheet from the hind limb skin of a female C57BL/6 mouse showing LCs stained for ATPase activity. *Bar* = 10 μm.

Langerhans Cells of Dorsal Ear Skin The LC density of ear epidermis in male C57BL/6 mice (465 ± 24/mm^2) was significantly less than that in females (542 ± 17/mm^2) (Table I). Although the density in BALB/c female mice was higher than that in males, the difference was not significant (males, 702 ± 17/mm^2; females, 809 ± 44/mm^2, Table I). In both strains, regularly spaced LCs of the ear had lower densities and longer dendrites than those in the hind limb skin. The male/female LC ratio was 0.86 for C57BL/6.

Effects of Castration Since sex differences in LC density were distinct in the hind limb skin of C57BL/6 mice, the effects of castration were examined in C57BL/6 males and females. The LC density in sham-operated males (12 weeks old) in the orchiectomy experiments was 564 ± 27/mm^2 (Table II) but in orchiectomized males, the density was significantly more (1179 ± 49/mm^2) (Table II). In ovariectomized females (1237 ± 52/mm^2), LC densities did not differ significantly from those in the sham-operated animals (1146 ± 49/mm^2) (Table II) at 4 weeks following the operation.

DISCUSSION

It is evident from the data presented above that mouse epidermal LC density shows sex differences since male mice had fewer LCs than females. From the castration experiments, it is suggested that the LC density in males may possibly be reduced by the testis.

A number of papers report LC density to show species and regional specificity and genetic variation without any indication of sex differences [10–12]. The LC density values reported in this paper agree with those previously reported. For example, the LC density in the hind limb and ear of male C57BL/6 mice (hind

Table I. Densities of Epidermal Langerhans Cells (LCs) in the Mouse

Region	Strain	Sex	LCs/mm^2 (mean ± SEM) (n)[a]
Hind limb	C57BL/6	Male	823 ± 20 (8)
		Female	1363 ± 52 (8)[b]
	BALB/c	Male	1689 ± 66 (8)
		Female	2249 ± 105 (8)[b]
Ear	C57BL/6	Male	465 ± 24 (8)
		Female	542 ± 17 (8)[c]
	BALB/c	Male	702 ± 17 (7)
		Female	809 ± 44 (8)

[a] n = Total number of animals.
[b] $p < 0.001$.
[c] $p < 0.02$.

Table II. Effects of Castration on the Densities of Epidermal Langerhans Cells (LCs) of the Hind Limb Skin in C57BL/6 Mice

Sex	Operation	LCs/mm^2 (mean ± SEM)(n)[a]
Male	Sham operation	564 ± 27 (6)
	Orchiectomy	1179 ± 49 (7)[b]
Female	Sham operation	1146 ± 49 (7)
	Ovariectomy	1237 ± 52 (9)

[a] n = Total number of animals.
[b] $p < 0.001$.

limb, 823 ± 20/mm^2; ear, 465 ± 24/mm^2) is in good agreement with that reported by Bergstresser et al (foot pad, 820/mm^2; ear, 470/mm^2) although they make no reference to the sex of their mice [4]. Our LC density for hind limb skin in BALB/c mice (males, 1689 ± 66/mm^2; females, 2249 ± 105/mm^2) also agrees with that reported by Mackenzie and Squier (up to 2000/mm^2) [15]. In this study, however, LC density at two body sites appeared to indicate sex differences for both mouse strains. This may be evident for the following reasons. (a) In this study, age-matched mice were used. Since in mice, as well as in humans [16], LC density decreases gradually with age (to be published elsewhere), data for age-unmatched animals would show considerable variance, thus making it difficult to detect sex differences. (b) As evident from Table I, indication of sex differences varies according to body site, being less in the ear skin often used for studies on LCs. Thus, detection of sex differences without systematic study of LC density in age-matched animals may be difficult.

Epidermal LCs are considered to give critical allosensitizing signals for graft rejection. They have been implicated also as antigen-presenting cells in the induction and expression of delayed contact hypersensitivity reactions [2,3] though there is a report in conflict with this view [17]. Our data strongly indicate that sex-matched animals should be used in studies on the functions of LCs. Based on the present findings, LC density is apparently reduced by the testis. Without such action exerted by this organ (in females or orchiectomized males), LC density increases. Thus, the higher LC density in females and castrated males may be one of the mechanisms responsible for higher rejection of skin allografts observed in females and orchiectomized males [7,8]. Dysfunctions of the testis induced by mutations or chemicals may also result in greater LC density and altered immune reactions in males.

The H-Y antigen is a Y chromosome-associated weak histocompatibility antigen expressed in males. The transplantation of skin grafts from males to females has facilitated the investigation of H-Y antigen expression. Rejection of H-Y incompatible skin grafts by females is known to show interstrain variation (reviewed in [18]). Recent evidence indicates that LCs possibly play important roles in the rejection of H-Y incompatible skin grafts [19]. Assuming that the rejection of H-Y incompatible skin grafts is influenced by sex differences in LC densities, the interstrain variation may be explained in part by strain variation of sex difference in LC densities.

Since the testis is a major source of sex hormones, reduction in LC density may possibly be caused by androgens. The following mechanisms may possibly be operative for the control of LC density. (a) Proliferation of epidermal LCs might be reduced by testosterone. Because LCs are known to have mitotic activity [20–22] and mitosis of thymocytes induced by calcium-independent mitogens has been reported to be inhibited by testosterone [23], it is conceivable that this proliferation is reduced by testosterone. (b) Langerhans cell migration has been reported in skin graft rejection [5], contact hypersensitivity [24], and wound healing [25]. Repopulation of the epithelium by LCs may possibly occur through this migration [26]. If testosterone has inhibitory effects on LC migration, a decrease in LC density should result.

Ovariectomy in the present study failed to have any significant effects on the LC density of hind limb skin. In contrast, LC density and morphology in the vaginal epithelium of the mouse is reported to be affected by ovariectomy or estrogen administration [27]. This discrepancy may be explained by the fact that the kinetics of the vaginal epithelium are controlled by estrogen, which may possibly have an indirect effect on LC density and morphology. A direct effect would suggest the possible existence of LC subpopulations differing in response to estrogen.

A comparison of LC shape and density of the ear skin and the hind limb skin in both sexes suggested the possibility that LC dendrite length increases with lessening density. Such a relationship between LC morphology and density has also been observed in vaginal epithelium with epithelial change in the estrous cycle and was suggested to relate to the immunologic role of LCs [28]. It may be reasonable to speculate that LCs, when the density has decreased, extend their dendrites to cover the epithelial tissue effectively. However, further experiments should be conducted to investigate functional differences between highly dendritic and stellate LCs.

The authors are grateful to Dr. M. Shamoto and Dr. K. Fukuyama for their valuable advice and critical reading of this manuscript.

REFERENCES

1. Langerhans P: Über die Nerven der menschlichen Haut. Virchows Arch [A] 44:325–337, 1868
2. Katz SI, Cooper KD, Iijima M, Tsuchida T: The role of Langerhans cells in antigen presentation. J Invest Dermatol 85 (suppl):96–98, 1985
3. Wolff K, Stingl G: The Langerhans cell. J Invest Dermatol 80 (suppl): 17–21, 1983
4. Bergstresser PR, Fletcher CR, Streilein W: Surface densities of Langerhans cells in relation to rodent epidermal sites with special immunologic properties. J Invest Dermatol 74:77–80, 1980
5. Streilein JW, Lonsberry LW, Bergstresser PR: Depletion of epidermal Langerhans cells and Ia immunogenicity from tape-stripped mouse skin. J Exp Med 155:863–871, 1982
6. Toews GB, Bergstresser PR, Streilein JW, Sullivan S: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 124:445–453, 1980
7. Castro JE: Immunological effects of orchidectomy. Br J Urol 47: 89–95, 1975
8. Graff RJ, Lappé MA, Snell GD: The influence of the gonads and adrenal glands on the immune response to skin grafts. Transplantation 7:105–111, 1969
9. Raveche ES, Klassen LW, Steinberg AD: Sex differences in formation of anti-T-cell antibodies. Nature 263:415–416, 1976
10. Berman B, Chen VL, France DS, Dotz WI, Petroni G: Anatomical mapping of epidermal Langerhans cell densities in adults. Br J Dermatol 109:553–558, 1983
11. Chen H, Yuan J, Wang Y, Silvers WK: Distribution of ATPase-positive Langerhans cells in normal adult human skin. Br J Dermatol 113:707–711, 1985
12. Wolff K, Winkelmann RK: Quantitative studies on the Langerhans cell population of guinea pig epidermis. J Invest Dermatol 48: 504–513, 1967
13. Baker KW, Habowsky EJ: EDTA separation and ATPase Langerhans cell staining in the mouse epidermis. J Invest Dermatol 80: 104–107, 1983
14. Chaker MB, Tharp MD, Bergstresser PR: Rodent epidermal Langerhans cells demonstrate greater histochemical specificity for ADP than for ATP and AMP. J Invest Dermatol 82:496–500, 1984
15. Mackenzie IC, Squier CA: Cytochemical identification of ATPase-positive Langerhans cells in EDTA-separated sheets of mouse epidermis. Br J Dermatol 92:523–533, 1975
16. Gilchrest BA, Murphy GF, Soter NA: Effect of chronologic aging

and ultraviolet irradiation on Langerhans cells in human epidermis. J Invest Dermatol 79:85–88, 1982

17. Baker D, Parker DD, Turk JL: Effect of depletion of epidermal dendritic cells on the induction of contact sensitivity in the guinea-pig. Br J Dermatol 113:285–294, 1985
18. Gasser DL, Silvers WK: Genetics and immunology of sex-linked antigens. Adv Immunol 75:215–247, 1972
19. Chen HD, Silvers WK: Influence of Langerhans cells on the survival of H-Y incompatible skin grafts in rats. J Invest Dermatol 81: 20–23, 1983
20. Czernielewski J, Vaigot P, Prunières M: Epidermal Langerhans cells—a cycling cell population. J Invest Dermatol 84:424–426, 1985
21. Gschnait F, Brenner W: Kinetics of epidermal Langerhans cells. J Invest Dermatol 73:566–569, 1979
22. Mackenzie IC: Labelling of murine epidermal Langerhans cells with H^3-thymidine. Am J Anat 144:127–136, 1975
23. Morgan JI, Hall AK, Perris AD: Requirements for divalent cations by hormonal mitogens and their interactions with sex steroids. Biochem Biophys Res Commun 66:188–194, 1975
24. Silberberg I, Baer RL, Rosenthal SA: The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pigs. J Invest Dermatol 66:210–217, 1976
25. Walsh LJ: Failure of Langerhans cells to repopulate gingival wounds *in vitro*. J Dent Res 62:643, 1983
26. Walsh LJ, Seymour GJ, Powell RN: The in vitro effect of retinol on human gingival epithelium. II. Modulation of Langerhans cell markers and interleukin-1 production. J Invest Dermatol 85:501–506, 1985
27. Young WG: Epithelial kinetics affect Langerhans' cells of mouse vaginal epithelium. Acta Anat (Basel) 123:131–136, 1985
28. Young WG, Newcomb GM, Hosking AR: The effect of atrophy, hyperplasia, and keratinization accompanying the estrous cycle on Langerhans' cells in mouse vaginal epithelium. Am J Anat 174: 173–186, 1985